Chicita F. CULBERSON* and William Louis CULBERSON*: The constituents of some species of the Thelotremataceae

チキタ・F・カルバーソン*・ウイリアム・L・カルバーソン*: 数種のチブサゴケ科地衣の含有成分

In the course of the preparation of a catalog of the natural products known from the lichens (C. F. Culberson, 1968) it was found that there were no reports upon the chemical constituents of any members of the family Thelotremataceae. This lack of information is surprising inasmuch as the Thelotremataceae is a large and widespread family. It contains several hundred described species, all of them crustose, and most of them confined to tropical and subtropical regions. Although the family seems to be an entirely natural one, its taxonomy is poorly understood. The present paper reports the chemical constituents of a few members of the family.

A preliminary survey of thelotremataceous specimens in Duke Herbarium indicated that those with a PD − thallus reaction contained primarily non-aromatic compounds, possibly triterpenes, which were not readily identifiable. None of the specimens contained well known PD − orcinol-type depsides or depsidones. Therefore, the present study concerns primarily the constituents of PD + specimens.

Microchemical Studies For the microchemical survey, 31 specimens representing 13 species were studied, namely the following:

1. **Ocellularia granulosa** (Tuck.) Zahlbr. — USA. Florida. Hernando Co.: Route 50, 3.7 mi. southwest of the Hernando-Sumter County line, on *Taxodium distichum*. *W. L. Culberson* and *C. F. Culberson* 10880 (DUKE). Highlands Co.: Highlands Hammock State Park, on *Sabal palmetto*. *B. J. Moore* 1064 (DUKE). Hillsborough Co.: Hillsborough River State Park. *B. J. Moore* 1338 (DUKE).
2. **Ocellularia** sp. — USA. Florida. Hillsborough Co.: Hillsborough River State Park. *B. J. Moore* 1337 (DUKE).
3. **Ocellularia** sp. — Costa Rica. Cartago: Platanillo, between La Suiza and Moravia, 3,000 ft., on a tree in the rain forest. *W. L. Culberson* 12433 (DUKE) (10 individuals of this collection were tested chemically).

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4. **Ocellularia** sp. — Costa Rica. Cartago: Platanillo, 3,000 ft., on a tree in the rain forest. *W. L. Culberson* 12909 (DUKE).

5. **Ocellularia** sp. — New Caledonia. Without precise locality. *Pionnier, s. n.* (DUKE). A specimen reported as *Thelotrema platycarpoides* Tuck. by Harmand (1912).

6. **Thelotrema piluliferum** Tuck. — USA. Hawaii. Oahu: Konahuanui, above Manoa. *A. A. Heller* 2272 (US).

7. **Thelotrema secernendum** Harm. — New Caledonia. Without precise locality. *Pionnier, s. n.* (3 syntype specimens, DUKE).

8. **Leptotrema** cfr. **glaucescens** (Nyl.) Müll. Arg. — USA. Alabama: Mobile Co.: Mobile. *C. Mohr, s. n.* (US).—Florida. Without precise locality. *J. D. Smith* and *C. D. Austin* in 1878 (US). On *Magnolia*. *W. W. Calkins* in 1889 (US). Polk Co.: Peace Creek. *J. D. Smith* in 1880 (US).

9. **Leptotrema ravenelii** (Tuck.) Fink — USA. North Carolina. Craven Co.: Havelock, on *Quercus falcata*. *W. L. Culberson* 10367 (DUKE).—South Carolina. Santee Canal. *H. W. Ravenel*, as *Reliquae Tuckermanianae* 139 (2 specimens, DUKE).—Mexico. San Luis Potosí: Las Palmas. *C. G. Pringle* 231 (DUKE).

10. **Leptotrema** cfr. **wightii** (Tayl.) Müll. Arg. — USA. Florida. Citrus Co.: Yulee Sugar Mill, on a fallen tree. *B. J. Moore* 1006 (DUKE). Highlands Co.: Highlands Hammock State Park, on a dead tree. *W. L. Culberson* and *C. F. Culberson* 10999 (DUKE).—North Carolina. Polk Co.: Tryon. *H. A. Green*, as *Decades of North American Lichens* 200 (DUKE).—South Carolina. Without precise locality. *H. W. Ravenel*, as *Reliquae Tuckermanianae* 142 (DUKE).

11. **Leptotrema** sp. — New Caledonia. Without precise locality. *Pionnier, s. n.* (2 specimens, DUKE). These specimens were reported as *Thelotrema phaeosporum* Nyl. by Harmand (1912).

12. **Leptotrema** sp. — New Caledonia. Without precise locality. *Pionnier, s. n.* (3 specimens, DUKE). These specimens were reported as *Thelotrema phaeosporum* Nyl. by Harmand (1912).

13. **Leptotrema** sp. — Jamaica. St. Ann: North of Hollymount, Mt. Diablo, 2,700 ft. *H. A. Hespenheide* 866 and 1131 (2 collections, DUKE). St. Thomas: John Crow Mts., Johnson Mt. *C. Racine* in 1967 (DUKE).

Fragments of all samples tested were extracted in vials with benzene at room temperature and then with acetone at elevated temperature. The dried extracts were chromatographed on Merck SiO_2-F_{254} analytical-layer plates with the solvent system benzene-dioxane-acetic acid (90 : 25 : 4), and the spots were visualized with 10% H_2SO_4 and heat. Fumarprotocetraric acid, which gives a low R_f value with this solvent, was confirmed by chromatography in butanol-acetone-water (5 : 1 : 2). In addition, crystals were observed for most compounds; they were identified by treatment of portions of the residue with the standard microcrystal test solutions. The tests are summarized in Table 1.

Table 1. The compounds found as constituents of the species studied of the Thelotremataceae.

Compound	R_f (TLC)	Color (H_2SO_4) (TLC)	Confirmatory Tests
Fumarprotocetraric acid	0.05	gray	TLC (Butanol : acetone : water, 5 : 1 : 2), R_f 0.41
Hypoprotocetraric acid	0.29	blue	GE, GA*o*-T, GWPy, KK
Lichexanthone	0.67	faint yellow	GE
Norstictic acid	0.42—0.43	yellow	GA*o*-T, KK
Psoromic acid	0.38	brown	GE, GA*o*-T
Stictic acid	0.35	yellow-orange	GA*o*-T
Unknown A	0.10	orange	
Unknown B	0.44	yellow-orange	
Unknown C	0.44	very pale coloration	

The occurrence of psoromic acid in *Ocellularia granulosa* was verified by extraction of a pure sample of psoromic acid (see below) which was then used as a standard in the microchemical tests on other specimens. Attempts to confirm, by microcrystal tests, the presence of psoromic acid indicated by thin-layer chromatography of the sample of an *Ocellularia* (species number 3 in the list above) also containing hypoprotocetraric acid and lichexanthone failed. But a preparative-layer chromatogram allowed the separation of sufficient psoromic acid for this species to give the crystal tests in the GE and GA*o*-T solutions. Tests on ten fragments from the sample of this *Ocellularia* showed that psoromic acid has a variable concentration and it was not detectable in two of the fragments.

The unknown B occurs only with stictic acid. It shows a spot on thin-layer chromatograms nearly identical to that of norstictic acid but it has a lesser tendency to trail and a slightly different color with the H_2SO_4 spray and heat. Microcrystal tests on a sample separated by preparative-layer chromatography and elution from the silica gel showed that this compound is not norstictic acid. It gives colorless needles (ϕ about 20°) in clusters in the GA*o*-T solution, and no crystals in the KK solution. The R_f value in n-hexane-ethyl ether-formic acid (5 : 1 : 4) solvent is also nearly identical to that of norstictic acid.

The unknown A was also extracted from the silica gel after preparative-layer chromatography. The only crystals observed for this material were

Table 2. The chemical constituents of 13 species of the Thelotremataceae and the number of specimens of each species tested. For the geographic origins of the specimens, see the correspondingly numbered list of species in the text.

	Hypoprotocetraric	Norstictic	Psoromic	Fumarprotocetraric	Stictic	Unknown A	Unknown B	Unknown C	Lichexanthone	Unknown pigment	Number tested
1. *Ocellularia granulosa*			+								3
2. *Ocellularia* sp.			+								1
3. *Ocellularia* sp.	+		±						+		1
4. *Ocellularia* sp.			+								1
5. *Ocellularia* sp.		+									1
6. *Thelotrema piluliferum*			+								1
7. *Thelotrema secernendum*				+							3
8. *Leptotrema* cfr. *glaucescens*					+	+	+				4
9. *Leptotrema ravenelii*										±	4
10. *Leptotrema* cfr. *wightii*					+	+	+				4
11. *Leptotrema* sp.					+	+	+				2
12. *Leptotrema* sp.					+	+		+			3
13. *Leptotrema* sp.					+	+					3

small needles ($\phi=0°$), singly or in clusters in the GE solution. The samples tested microchemically and containing unknowns A and B were too small for further tests.

Unknown C has the same R_f value as unknown B in the benzene-dioxane-acetic acid solvent. Its spot on the chromatogram is distinguished from that of unknown B by its pronounced fluorescence in ultraviolet light, its pale coloration with 10% H_2SO_4 and heat, and its larger size and more oval shape. A preparative-layer separation showed that unknown C is not norstictic acid.

The results of the microchemical study are given in Table 2.

Macroextraction of *Ocellularia granulosa* The abundant material of *Ocellularia granulosa* for the macroextraction came from Hernando County, Florida, the first collection listed under species number 1 above. The thin, tightly-adhering crustose thallus could not be separated from the bark. Thin shavings of air-dry bark and lichen totaling 129 g were soaked three times over a period of two days in anhydrous, peroxide-free ethyl ether. Each extraction was heated briefly before it was filtered from the lichen. The combined extracts were evaporated to dryness at diminished pressure and moderate temperature (40°). The reddish solid (3.8 g, 2.9%), probably discolored by pigments from the bark contaminant, was washed with benzene (30 ml), then with chloroform (20 ml), and finally with acetone (15 ml). The nearly white solid recrystallized from acetone as beautiful needles (0.912 g, 0.71%). A sample was recrystallized again from acetone (Calcd. for $C_{18}H_{14}O_8$: C, 60.34; H, 3.94. Found: C, 60.35; H, 3.96%), m. p. 268.5-269°; reported by Asahina and Hayashi (1933), m. p. 265°d with coloring at 262°. The infrared spectrum (nujol mull) of anhydrous psoromic acid shows maxima at 3150 and 3080 (OH), 1740 (depsidone C=O), 1650 (bonded C=O), and 1620 and 1580 (benzenoid C=C) cm^{-1}. The ultraviolet spectrum (95% ethanol) shows λ_{max} (log ε) at 240 (4.49), λ_{sh} (log ε) at 270 (4.11), and λ_{min} (log ε) at 225 (4.38) mμ. Psoromic acid crystallizes as a monohydrate from acetone or ethanol containing water (Calcd. for a monohydrate: loss of weight on drying, 5.02%. Found: 4.97%). The monohydrate shows maxima in the infrared (nujol mull) at 3500 and 3400 (OH), 2600 (carboxylic acid OH), 1750 (depsidone C=O), 1720 (carboxylic acid C=O), 1650 (bonded aldehyde C=O), and 1585 and 1565 (benzenoid C=C) cm^{-1}.

A sample of psoromic acid (207 mg) in 95% ethanol (75 ml) was reduced over 10% palladium on carbon at room temperature and atmospheric pressure. The filtered solution was concentrated somewhat and the hypopsoromic acid monohydrate (156 mg, 75%), which precipitated upon dilution with water, was recrystallized from acetone. Anhydrous product was obtained by drying the sample in a vacuum at 110° over P_2O_5. The compound did not melt; it showed some thermally induced transformation from 150–165° and charred slowly above 230° (reported by Asahina and Hayashi (1933), coloring at 160° and decomposition at 230°). Hypopsoromic acid monohydrate shows maxima in the infrared (nujol mull) at 3040–3650 (OH), 2475–2750 (carboxylic acid OH), 1695 (bonded C=O), and 1610 and 1585 (benzenoid C=C) cm^{-1}. In the ultraviolet spectrum (95% ethanol) it shows λ_{max} (log ε) at 213 (4.63) and 275 (4.04) and λ_{min} (log ε) at 256 (3.94) mμ. The monohydrate lost 4.74% of its weight upon drying (Calcd. for a monohydrate: 4.97%; analysis by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., USA). A nuclear magnetic resonance spectrum (60 MHz) of anhydrous product in acetone-d_6 gave signals at 397 (1 aromatic –H at position 1′), 376 (1 aromatic –H at position 5), 204 (1 $-OCH_3$), 110 (1 aromatic $-CH_3$), 105 (1 aromatic $-CH_3$), and 101 (1 aromatic $-CH_3$) Hz. Hypopsoromic acid was converted to the methyl ester with diazomethane in ethyl ether at 0°, and the product was recrystallized from acetone-water, m. p. 265.5–267.8°d. The methyl ester absorbs in the infrared (nujol mull) at 3260 (OH), 1750 (depsidone C=O), 1710 (methyl ester C=O), and 1620 and 1580 (benzenoid C=C) cm^{-1}. The ultraviolet spectrum (95% ethanol) shows maxima at 215 and 274 mμ.

Discussion The members of the Thelotremataceae synthesizing aromatic compounds characteristically produce β-orcinol depsidones rather than depsides or orcinol-type substances. The compounds found in the Thelotremataceae are very similar to the aromatic products of the Graphidaceae (Wirth & Hale, 1963), for example both families produce the β-orcinol depsidones stictic acid and norstictic acid and the yellow pigment lichexanthone. In addition, the β-orcinol depsidone fumarprotocetraric acid found in this study is closely related to protocetraric acid found in the Graphidaceae. Both families contain many species producing nonaromatic constituents. Many of these substances may be triterpenes, and the triterpene zeorin has already been found in the Graphidaceae. These chemical similarities support Santesson's (1952) observation that the Thelotremataceae and the Graphidaceae are very closely related taxonomically.

The structure of psoromic acid was studied extensively by Asahina and Hayashi (1928, 1933, 1937) and Hayashi (1937), and it was finally proven by Asahina and Shibata (1939). But dry distillation of psoromic acid yields, in addition to atranol, 3-hydroxy-5-methoxy-4-methylphthalic anhydride, which was explained by a salol-type rearrangement to a benzophenone with an *ortho*-carboxylic acid followed by cleavage to the anhydride. In 1963 Dean suggested that the formation of a phthalic acid would be better explained if psoromic acid were a β-coumaranone rather than a depsidone. The spectral evidence now available, however, entirely supports the structure proposed by Asahina and Shibata.

The infrared spectrum of psoromic acid shows a typical depsidone ester peak at 1740 cm^{-1}. Huneck and Linscheid (1968) found two aromatic protons in the nuclear magnetic resonance spectrum of psoromic acid and of its triacetate, and we find two aromatic protons in hypopsoromic acid. However, a β-coumaranone structure would require three protons, one in the aromatic ring and two in the dienone ring. The mass spectra of psoromic acid and of methyl 4-0-methyl-psoromate were recently reported (Huneck *et al.*, 1968).

The structure of psoromic acid suggests a further extension of the oxidation process observed in norstictic and stictic acids, accompanied by decarboxylation and 2′-0-methylation. Hypoprotocetraric acid is, on the other hand, the most reduced β-orcinol depsidone possible. Although psoromic acid and hypoprotocetraric acid occur together in one *Ocellularia* species, their structures are too distinct to support a very close biosynthetic connection between them. Psoromic acid is a relatively widespread compound, but it has not previously been found in the same lichen with hypoprotocetraric acid although it has been found in *Ramalina*, the only genus from which hypoprotocetraric acid was known prior to the present report.

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主にデューク大学に保存されている標本を使い，従来全く報告されたことのないチブサゴケ科地衣の化学成分を調査して，その結果を第2表に示した。また，*Ocellularia granulosa* の多量の標本から成分を抽出し，その抽出物について赤外線および紫外線分光分析や核磁気共鳴 (NMR) を行なってプソローム酸の存在を確認し，またプソローム酸の構造式については，朝比奈・柴田 (1939) の提唱したものが妥当と認められることを述べた。また，チブサゴケ科地衣の化学成分は β-オルチノール・デプシドンを主とし，ほかにトリテルペンを含むことから，モジゴケ科と近縁であることを指摘した。